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Diet-induced obesity causes ghrelin resistance in reward processing tasks

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ABSTRACT

Diet-induced obesity (DIO) causes ghrelin resistance in hypothalamic Agouti-related peptide (AgRP) neurons. However, ghrelin promotes feeding through actions at both the hypothalamus and mesolimbic dopamine reward pathways. Therefore, we hypothesized that DIO would also establish ghrelin resistance in the ventral tegmental area (VTA), a major site of dopaminergic cell bodies important in reward processing. We observed reduced sucrose and saccharin consumption in Ghrelin KO vs Ghrelin WT mice. Moreover, DIO reduced saccharin consumption relative to chow-fed controls. These data suggest that the deletion of ghrelin and high fat diet both cause anhedonia. To assess if these are causally related, we tested whether DIO caused ghrelin resistance in a classic model of drug reward, conditioned place preference (CPP). Chow or high fat diet (HFD) mice were conditioned with ghrelin (1 mg/kg in 10 ml/kg ip) in the presence or absence of food in the conditioning chamber. We observed a CPP to ghrelin in chow-fed mice but not in HFD-fed mice. HFD-fed mice still showed a CPP for cocaine (20 mg/kg), indicating that they maintained the ability to develop conditioned behaviour. The absence of food availability during ghrelin conditioning sessions induced a conditioned place aversion, an effect that was still present in both chow and HFD mice. Bilateral intra-VTA ghrelin injection (0.33 μg/μl in 0.5 μl) robustly increased feeding in both chow-fed and high fat diet (HFD)-fed mice; however, this was correlated with body weight only in the chow-fed mice. Our results suggest that DIO causes ghrelin resistance albeit not directly in the VTA. We suggest there is impaired ghrelin sensitivity in upstream pathways regulating reward pathways, highlighting a functional role for ghrelin linking appropriate metabolic sensing with reward processing.

1. Introduction

Ghrelin is a 28 amino acid peptide secreted from the stomach to signal negative energy balance. It is the endogenous ligand of the growth hormone secretagogue receptor (GHSR1a) (Andrews, 2011), but must be esterified with an acyl group for full efficacy at this receptor. It is the only known circulating factor that increases food intake and has a well-described role in promoting feeding through hypothalamic circuits (Andrews, 2011). The GHSR1a is highly expressed in the hypothalamus with highest levels in the arcuate nucleus (Zigman et al., 2006), where it is colocalised with >90% of neuropeptide Y (NPY) neurons (Willesen et al., 1999). Ghrelin's ability to increase food intake requires Agouti-related peptide (AgRP) and NPY neurons, as ablation of AgRP neurons prevents ghrelin-induced food intake (Luquet et al., 2007).

The GHSR1a is also found on midbrain dopamine neurons in the substantia nigra (Andrews et al., 2009) and ventral tegmental area (VTA) (Abizaid et al., 2006). The cell bodies of dopamine neurons located in the VTA form a key component of the brain reward circuitry and ghrelin injected into the VTA or nucleus accumbens elicits food intake (Abizaid et al., 2006; Naleid et al., 2005). Re-expression of GHSR1a only on tyrosine hydroxylase (TH) neurons, a population that includes dopamine neurons, partially restores ghrelin-induced food intake (Chuang et al., 2011), suggesting that the ability of ghrelin to fully induce food intake requires both GHSR1a signalling in arcuate (ARC) AgRP neurons and TH neurons, the latter presumably via reward processing. Behavioural and physiological evidence shows that ghrelin plays a key role in reward processing. Exogenous ghrelin administration increases appetitive drive or motivated responding in a number of

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behavioural paradigms including progressive ratio response tasks (Skibicka et al., 2011). Conversely, lack of ghrelin signalling disrupts behavioural responses to other drugs such as cocaine, nicotine and alcohol (Abizaid et al., 2011; Jerlhag et al., 2010, 2009). These findings indicate that ghrelin is critical for processing of reward information.

Conditioned place preference/aversion (CPP/CPA) is a commonly used behavioural task to assess the rewarding or aversive nature of particular stimuli. It has been used extensively to test the motivational properties of drugs of abuse in rodents (Tzschentke, 2007). There is considerable literature around ghrelin's ability to potentiate the effectiveness of the unconditioned stimulus in the CPP test. Ghrelin given in conjunction with cocaine increases the strength of the preference for the paired compartment (Davis et al., 2007), whereas GHSR1a antagonism attenuates CPP to nicotine (Jerlhag and Engel, 2011), cocaine, and amphetamine (Jerlhag et al., 2010). Ghrelin knockout mice also show deficits in forming a CPP in response to ethanol (Bahi et al., 2013). Ghrelin given during the conditioning days of a CPP protocol, or on the test day only, promotes a conditioned place preference in response to a high fat diet pellet (Perello et al., 2010). Others have shown that ghrelin alone, at relatively low doses, is able to condition a place preference (Jerlhag, 2008).

Our group previously demonstrated that the hypothalamus becomes resistant to ghrelin signalling after periods of high fat diet (HFD) feeding, which is accompanied by a pronounced functional collapse in the NPY/AgRP neurons of the ARC (Briggs et al., 2010). These deficits occur after several weeks of HFD feeding (Briggs et al., 2014), and persist while body weight remains high, but can be reversed with diet-induced weight loss (Briggs et al., 2013). Others have recently demonstrated ghrelin resistance in the vagal afferents and nodose ganglion, suggesting that this phenomenon is not limited to the hypothalamus (Naznin et al., 2015). The interaction between HFD feeding and reward processing is as yet not well understood, however ghrelin resistance appears to extend to behavioural measures beyond feeding. In chow fed rats, ghrelin increases responding on a progressive ratio task, however it is ineffective in altering responding in obese rats (Finger et al., 2012). It is currently unclear exactly how HFD feeding effects other ghrelin-responsive cell populations, such as those in the VTA, and whether this has functional effects for mouse behaviour and reward processing.

In this study we aimed to examine how HFD affects the role of ghrelin in reward processing. We hypothesised that ghrelin resistance would be present in the VTA, and that this would produce a number of deficits in behavioural tasks associated with reward processing and consolidation.

2. Methods

2.1. Mice and housing

Male C57Bl/6J mice were obtained at 9–10 weeks old from Monash Animal Services, Victoria, Australia, and were group housed (4 per cage) under controlled conditions (21 °C and 12:12 h light/dark cycle) and maintained on chow diet or HFD with free access to food and water. The chow diet (rat and mouse pellets, specialty feeds, Western Australia) contained 20% protein, 4.8% total fat, 4.8% crude fibre, and 14 kJ/g of digestible energy. The HFD (SF04-001, specialty feeds, Western Australia) contained by weight: 22.6% protein, 23.5% fat, 20% sucrose, 20% starch, 5.4% crude fibre with an energy content of 19 kJ/g. Experiments were conducted in accordance with the Monash University Animal Ethics Committee guidelines. For experiments undertaken at time points on HFD, mice were trained during the last two weeks of HFD feeding. Ghrelin knock out mice (ghrelin KO) and wildtype littermates (ghrelin WT) were obtained from Regeneron Pharmaceuticals and back crossed to C57Bl/6J. Mice were bred at Monash Animal Services and housed as above.

2.2. Drugs

Ghrelin (PolyPeptide group, Strasbourg, France) and cocaine (Glaxo Australia, Pty Ltd.) were given by intraperitoneal injection, in a volume of 10 ml/kg, dissolved in isotonic saline. Isotonic saline acted as the vehicle for all injections. A dose of 1 mg/kg was chosen for intraperitoneal ghrelin administration following a dose response (Fig 1A), as this dose gave a large and reliable increase in food intake. Intra VTA ghrelin was given bilaterally at 0.5 μg in a volume of 0.5 μl. Cocaine was given at a dose of 20 mg/kg, as previous experience indicated this dose reliably conditions a place preference in C57black/6 mice (Bird et al., 2014; McPherson et al., 2010). All drugs were dissolved fresh on day of administration.

Sucrose (CSR, Australia) and Saccharin (Sigma, Australia) were dissolved in tap water at concentrations of 10% and 0.1% (w/v), respectively. These concentrations were used as they reliably increase fluid consumption.

2.3. VTA cannulations

Mice were anaesthetised using isoflurane (5% induction, 2% maintenance in oxygen) and 26 G bilateral cannulae (Plastics One Inc., Roanoke, VA, USA) aimed at the VTA (coordinates relative to bregma in mm; anterior posterior = −3.2, medial lateral = 0.6, dorsal ventral = −4.6) were implanted. Cannulae were fixed to the scull using light-curing dental cement (G-bond and G-aenial Universal Flo, GC, Henry Schein Halas). Following surgery, mice were given metacam as a post-operative analgesic and allowed at least 7 days recovery before injections commenced. Cannula placement was histologically verified post mortem.

We performed a dose response and identified 0.33 μg/μl as the lowest effective dose of ghrelin in the VTA to elicit food intake, this was subsequently used for all feeding studies in a 0.5 μl injection volume.

2.4. Sucrose and saccharin two bottle choice

Mice were trained for 4 days in the two bottle choice test, and then consumption was measured on day 5. Mice were water deprived overnight before first exposure. Mice were removed from their group-housed home cage and placed individually in fresh cages without bedding, and two bottles in the cage lid, one with water and one with sweetener (either 10% w/v sucrose or 0.1% w/v saccharin) and allowed 90 min to consume from either bottle, then returned to group housing. This procedure was repeated with water restriction on day 2, and without water restriction on days 3 and 4, with bottles alternating sides on each day. On test day, bottles were randomly assigned a side and consumption in the 90 min window was measured for both bottles.

2.5. CPP methods

Conditioned place preference testing was performed in custom-made boxes. Boxes were constructed of plexiglass and contained two side zones (28 cm × 21 cm) and a middle zone (21 cm × 9 cm), with the middle zone being divided from the end zones by opaque white plexiglass walls. Each end zone was differentiated by wall pattern and floor texture. The middle compartment contained button lights fixed to the wall to increase illumination and discourage mice from spending time there during testing. Light levels were 19 lumens in the centre compartment and 5.25 lumens in the

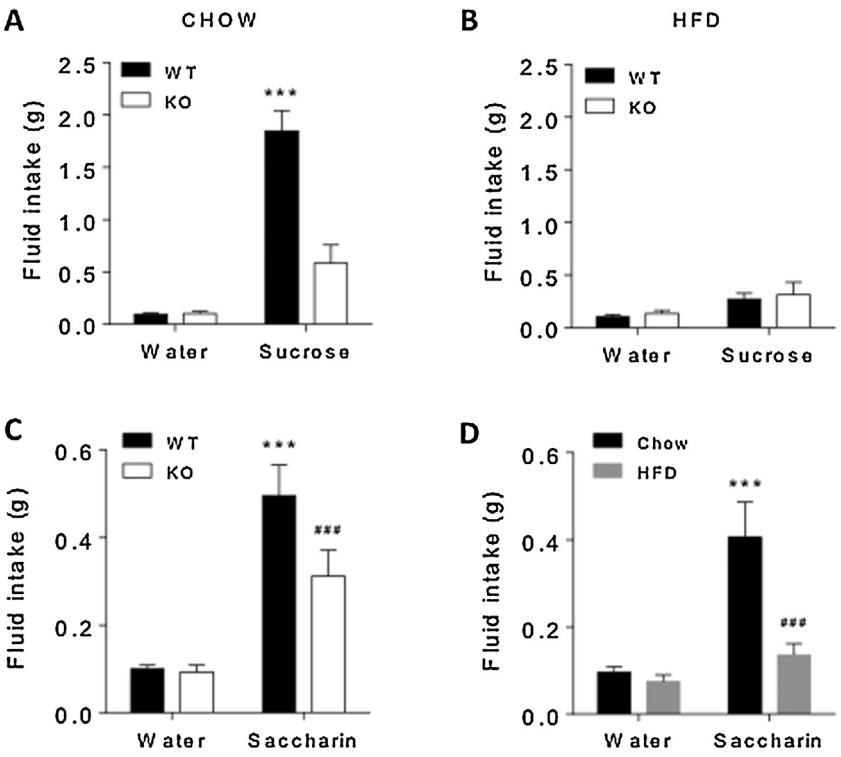

Fig. 1. (A) 10% (w/v) sucrose solution consumption is significantly reduced in ghrelin KO mice relative to ghrelin WT mice (B) after 3 weeks of high fat diet consumption this difference is abolished. (C) 0.1% (w/v) saccharin solution consumption is significantly reduced in ghrelin KO mice relative to ghrelin WT mice. (D) 0.1% saccharin preference is significantly reduced in C57black/6 mice on a HFD for 3 weeks. ***$p < 0.001$ compared to WT + sucrose or saccharin; ***$p < 0.01$ compared to chow + saccharin; two way ANOVA followed by Bonferroni comparison.

end zones. Familiarisation trials were filmed and scored for initial preference. Mice spending between 45% and 55% spent on either side were considered to have no initial preference (unbiased). Any mouse spending <45% was allocated a conditioning side in a biased manner, pairing the test treatment with the non-preferred side. Typically fewer than 30% of mice fitted into this category. For ghrelin conditioning, ghrelin or saline were administered IP on alternating days for 11 days (6 ghrelin days, 5 saline days). Conditioning sessions lasted 20 min. In all conditioning trials mice were injected with either ghrelin or saline and placed back in their home cage for no more than 5 min, then placed in the conditioning boxes. Cocaine conditioning occurred for 7 days (4 cocaine days, 3 saline days). Mice were injected with cocaine and placed in the conditioning chamber for 20 min. The preference score equals the time spent in the specified conditioning compartment divided by the total time spent in both compartments, excluding the middle neutral zone, multiplied by 100 (Brown et al., 2009). All trials were filmed and later scored, by an experimenter blinded to the treatment groups, using Ethovision software (Ethovision XT; Noldus Information Technology; Wageningen, The Netherlands) to electronically track mice and generate scores. The compartments were thoroughly washed with mild detergent in warm water between trials.

2.6. Statistics

All data are reported as the mean ± sem. Student's unpaired *t*-tests were used to assess differences in body weight. All other data sets were examined using a one-way or two-way ANOVA with post hoc analysis as indicated in the text.

3. Results

We first investigated sucrose solution consumption in Ghrelin KO mice. When eating a chow diet, ghrelin KO mice had significantly reduced consumption of a 10% sucrose solution compared to WT littermates (Fig 1A; $F(1,36) = 23.09$, $p < 0.001$). Sucrose solution consumption was measured again in the same mice after three weeks of HFD feeding, at which time there was no longer a significant difference in consumption between WT and KO mice (Fig 1B; $F(1,33) = 0.263$, $p > 0.05$). Considering caloric content contributes to the rewarding properties of sucrose, we next sought to examine whether removal of caloric content lead to similar results by using saccharin. Chow fed ghrelin KO mice also had significantly reduced saccharin solution consumption compared to WT mice (Fig 1C; $F(1,40) = 4.616$, $p < 0.05$). Similarly, HFD itself caused anhedonia, as shown in Fig 1D, where a 3-week HFD exposure caused reduced saccharin solution consumption relative to chow-fed wild-type mice ($F(1,34) = 15.87$, $p < 0.001$). These results suggest that ghrelin is involved in the normal processing of hedonic responses to saccharin or sucrose and that the loss of ghrelin signalling due to ghrelin resistance in DIO mice may contribute to the anhedonia noted in HFD vs chow fed mice.

To investigate if the 3 weeks of HFD causes ghrelin resistance in reward pathways, we designed experiments to test the function of ghrelin on well-defined condition place preference behaviour in mice fed chow or HFD. We selected a dose of 1 mg/kg based on a dose response study (Fig 2A $F(3) = 8.622$, $p < 0.001$), as this dose reliably and potently induced eating. Three weeks of HFD feeding produced significantly heavier mice in the HFD group (Fig 2B, $t(46) = 6.763$, $p < 0.001$), and these mice also had significantly worse glucose tolerance (Fig 2C, $F(2,17) = 27.09$, $p < 0.001$).

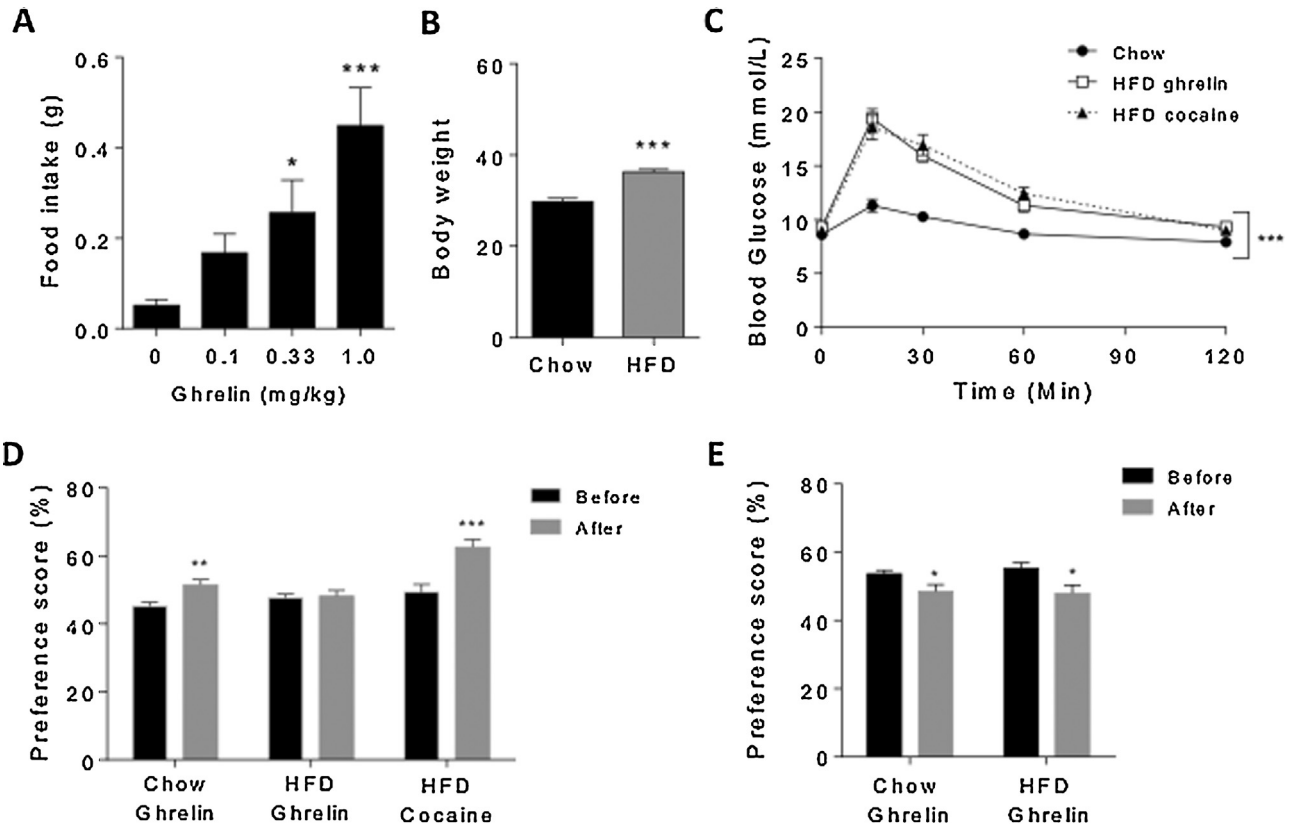

Fig. 2. (A) Ghrelin dose response in chow fed C57black/6 mice, $p < 0.05$, $^{***}p < 0.001$, one way ANOVA. (B) Three weeks of HFD feeding results in significantly increased body weight, $^{***}p < 0.001$, *t* test and (C) significantly worsened glucose tolerance $^{***}p < 0.001$, main effect of diet 2 way RM ANOVA. Conditioned place preference scores for conditioned compartment before and after conditioning, (D) with food present during conditioning and on test day, and (E) with food absent during conditioning and on test day. $^{*}p < 0.05$ $^{***}p < 0.01$ $^{***}p < 0.001$, 2 way RM ANOVA, Sidak's multiple comparison.

When mice were conditioned with food present in the conditioning boxes, ghrelin (1 mg/kg ip) produced a significant shift in preference for the conditioned side relative to pre-conditioning (Fig 2D, $F(2,64) = 12.3$, $p < 0.01$, Sidak's multiple comparison test). This effect was absent in HFD-fed mice conditioned with ghrelin, suggesting that HFD prevents the ability of ghrelin to condition a place preference (Fig 2D). We included a group of HFD fed mice conditioned with cocaine to ascertain whether these mice could form a CPP given an appropriate unconditioned stimulus. This group showed a strong shift in preference for the cocaine-paired side, relative to pre-conditioning (Fig 2D, Sidak's multiple comparison test), indicating that HFD mice are capable of forming a CPP. We repeated the experiment without food in the conditioning boxes during conditioning trials, and observed the opposite effect of ghrelin on preference. Chow fed mice treated with ghrelin in the absence of food showed a significant aversion for the conditioned side relative to pre-conditioning, and interestingly this effect was preserved in mice on a HFD (Fig 2E, $F(1,36) = 14.44$ $p < 0.01$).

Our data indicated DIO induces ghrelin resistance in pathways regulating a CPP but not a CPA. To test whether DIO causes direct ghrelin resistance in the VTA, we measured food intake after intra-VTA ghrelin injection as a proxy for ghrelin action on VTA dopamine neurons. Administration of ghrelin directly into the VTA increased feeding in chow fed and HFD mice over a two hour period (Fig 3A, $F(2,68) = 18.20$, $p < 0.001$,), suggesting a lack of ghrelin resistance to intra-VTA ghrelin in HFD mice. To investigate this further we used linear regression to correlate each animal's ghrelin-induced food intake with its body weight on a chow or HFD. There was no correlation between intra-VTA aCSF food intake in chow or HFD mice (Fig. 3B, $r^2 = 0.04$ and 0.05, respectively). Intriguingly, we observed a correlation between body weight and food intake in chow fed mice injected with ghrelin into the VTA (Fig 3C, $r^2 = 0.38$). This relationship was absent in mice eating a HFD diet (Fig. 3C, $r^2 = 0.03$). These results suggest that although HFD did not impair intra-VTA ghrelin-induced food intake relative to chow animals, the ability of HFD mice to sense and integrate metabolic information and coordinate an appropriate feeding response was compromised.

4. Discussion

We previously described that HFD exposure for 12 weeks induces ghrelin resistance in hypothalamic NPY/AgRP neurons (Briggs et al., 2010). Subsequent time course studies show that this ghrelin resistance occurs after just 3 weeks exposure to HFD (Briggs et al., 2014) and that ghrelin resistance was reversible after diet-restricted weight loss (Briggs et al., 2013). In this study, we determined if HFD causes ghrelin resistance in the VTA, a key node for reward processing.

We report 4 key findings; (1) HFD does not affect the ability of intra-VTA ghrelin to increase food intake, although it does interfere with appropriate portion control; (2) HFD prevents a ghrelin-induced CPP, as observed in chow mice; (3) the ability of ghrelin to condition a place preference depends on the presence of food during the conditioning period and (4) in the absence of food ghrelin is a physiological signal that encodes negative valence and leads to a conditioned place aversion. Collectively, these data support the idea that HFD causes ghrelin resistance in extra-hypothalamic pathways regulating reward behaviour.

Intra-VTA delivery of ghrelin increases feeding and operant responding for sucrose in chow fed animals, showing that ghrelin targets VTA dopamine neurons to increase food intake (Abizaid et al., 2006; Naleid et al., 2005). This is supported by genetic studies

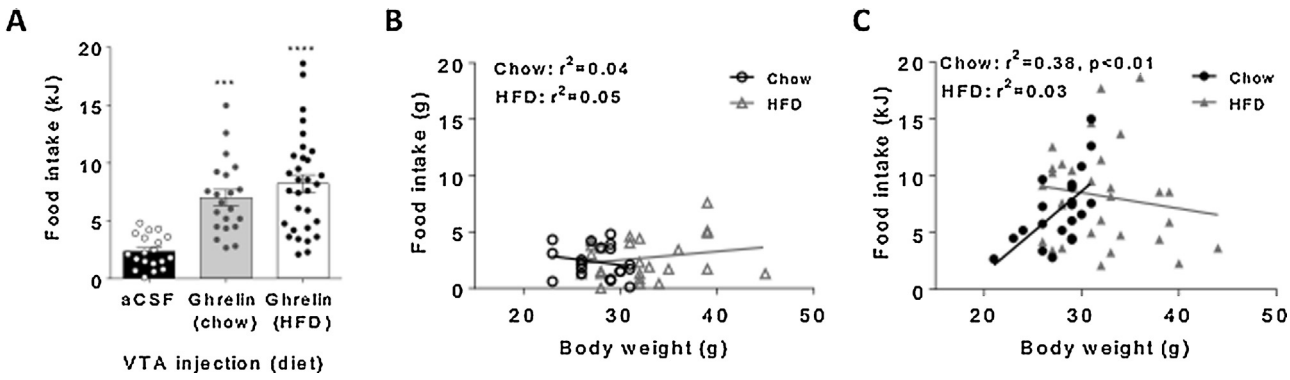

Fig. 3. (A) Intra-VTA ghrelin injection increases food intake in both chow and HFD-fed mice. (B) No correlations were observed in mice injected with aCSF into the VTA. (C) Body weight correlates with the intra-VTA ghrelin-induced feeding response in chow but not HFD mice, linear regression analysis.

whereby restoring GHSRs only in TH-expressing neurons partially restores feeding behaviour (Chuang et al., 2011). Interestingly, in the present study intra-VTA ghrelin robustly increased food intake in mice on both a chow and HFD suggesting HFD *per se* does not cause ghrelin resistance directly in the VTA. However, intra-VTA injection represents a non-physiological source of exogenous ghrelin into the VTA and studies show that peripheral ghrelin access into the brain is largely limited to the hypothalamus (Cabral et al., 2013, 2014). When we performed linear regression analysis we observed a clear correlation between intra-VTA ghrelin-induced feeding and body weight in chow, but not HFD mice. These results point towards a relationship between functional sensing of metabolic state (body weight) and intra-VTA ghrelin responsiveness. Since intra-VTA ghrelin increases food intake equally in both chow and HFD fed mice, we posit that the ability to sense metabolic state (body weight) is regulated in upstream neural circuits that regulate VTA dopaminergic pathways.

One potential upstream neural target may be hypothalamic AgRP neurons. Firstly, AgRP neurons influence dopamine-related behaviours unrelated to feeding, such as cocaine responsiveness (Dietrich et al., 2012) and secondly, AgRP neurons increase motivation to obtain food and food seeking behaviour (Krashes et al., 2011). Recent evidence suggests that direct activation of AgRP neurons can encode a negative valence signal and acts as a negative reinforcer, while silencing these neurons in the face of hunger is rewarding (Betley et al., 2015). As AgRP neurons are the canonical target of ghrelin, our finding support the idea that ghrelin-induced activation of AgRP neurons in the absence of food may act in a similar way to condition the aversion seen in the current experiments. Given HFD causes ghrelin resistance in AgRP neurons (Briggs et al., 2010), we hypothesise that this impairs the ability of AgRP neurons to communicate metabolic state with dopamine reward systems in the VTA. This suggestion requires further experimental evidence.

CPP is a behavioural task based on the association of an internal reward state established by an exogenous treatment (i.e. ghrelin in our experiments) and a context. The ability of an animal to form a conditioned place preference to drugs of reward such as cocaine, amphetamine, morphine, alcohol and heroin requires a functional mesolimbic dopamine system (Chen et al., 2006; Hoffman and Beninger, 1989; Spyraki et al., 1987; Walker and Ettenberg, 2007). Increased ghrelin levels potentiate the motivational effects of a number of substances, including cocaine, alcohol and amphetamine (Dickson et al., 2011). Our data support previous studies showing that ghrelin induces a CPP by regulating the mesolimbic dopamine system (Jerlhag, 2008). Peripheral or central ghrelin in mice promotes dopamine availability in the nucleus accumbens, while antagonism of the GHSR reduces drug-induced dopamine release (Abizaid et al., 2006; Jerlhag et al., 2007; Jerlhag and Engel, 2011). Therefore the inability of ghrelin to induce a CPP in HFD mice supports the idea the ghrelin resistance occurs in pathways controlling reward behaviours, including non-dopaminergic inputs. It is important to note that this is not just a phenomenon associated with specific CPP behaviour as ghrelin resistance has also been observed in operant based motivational tasks in diet-induced obese rats (Finger et al., 2012).

The rewarding effects of cocaine and amphetamine are diminished following high fat diet feeding (Davis et al., 2008; Wellman et al., 2007). It remained possible that HFD would cause an attenuated conditioned response; therefore we used cocaine to confirm that HFD mice were still capable of forming a conditioned place preference. Our results show that mice retain the ability to form a conditioned response to cocaine with HFD feeding, strongly indicating that our conditioning and testing protocol is robust and valid.

One of the most striking observations from our study is that the ability of ghrelin to induce a CPP depends on food availability during the conditioning sessions, as the absence of food led to a conditioned place aversion. A similar phenomenon has been observed with locomotor behaviour, as ghrelin initiates locomotor behaviour in the absence of food but not in the presence of food (Jerlhag et al., 2006; Tang-Christensen et al., 2004). The increase in locomotor behaviour is interpreted as food-seeking behaviour and acute activation of AgRP neurons, the key neural target for ghrelin-induced food intake, increases locomotor behaviour in the absence but not presence of food. AgRP neurons convey the sense of hunger and recent evidence highlights that AgRP activation in the absence of food intake can lead to repetitive complex behaviours that are reversed by food consumption (Dietrich et al., 2015). Thus, the presence or absence of food after ghrelin injection elicits distinctive opposing behavioural patterns. As noted above, remote activation of AgRP neurons encodes a negative valence signal that produces a CPA and silencing AgRP neurons in fasted mice produces a CPP (Betley et al., 2015). Our results produced with ghrelin administration show the same observations and suggest that plasma ghrelin may be the physiological signal that links metabolic state (hunger) with the negative valence encoded by artificial activation of AgRP neurons (Betley et al., 2015).

Intriguingly there also appears to be a dual role of ghrelin in anxiety-like behaviour with ghrelin suppressing anxiety in stressful situations but not during non-stressful conditions (Spencer et al., 2012). Moreover, acute ghrelin appears to drive anxiety whereas chronic ghrelin reduces anxiety, (for review (Spencer et al., 2015)). Thus, we believe that conditioning animals to ghrelin in the absence of food may acutely drive an aversive internal state, perhaps due to anxiety from an excessive hunger signal, leading to the formation of a CPA. The most remarkable aspect of the ghrelin-induced CPA in the absence of food is that it is not affected by diet-induced

obesity, as both chow and HFD mice show a CPA under these conditioning parameters. This suggests the possibility that different neural circuits controlling ghrelin-induced CPP or CPA exist. The description that different subpopulations of hunger-sensitive AgRP neurons drive feeding or non-feeding related behaviours (Betley et al., 2013) together with evidence that AgRP neurons drive repetitive stereotypic behaviour independent of hunger state (Dietrich et al., 2015) support this idea.

Our results suggest that ghrelin KO are anhedonic based on preference for saccharin or sucrose, indicating that calorie content does not affect the behavioural output. Overdiun et al. showed that ghrelin strongly promotes motivated behaviour but does not seem to affect the "pleasantness" of taste stimuli (Overduin et al., 2012). However, a number of previous studies show ghrelin increases cocaine effectiveness (Davis et al., 2007; Wellman et al., 2008), and that mice lacking ghrelin signalling show deficits in their responses to nicotine or alcohol (Jerlhag et al., 2009, 2011). Collectively, our saccharin and sucrose data together with the results above suggest ghrelin acts an amplifier of other pleasurable stimuli rather than being intrinsically rewarding itself. HFD-fed mice had a similar disinterest in the sucrose and saccharin solutions, which points toward ghrelin resistance being a driver of this reduced hedonic tone. It is likely, however, that the reduction in preference for sweet taste following HFD exposure is multifactorial.

In summary, our results suggest ghrelin resistance occurs in pathways regulating CPP behaviour as an index of reward processing. However these data do not support direct ghrelin resistance on dopamine neurons in the VTA since the feeding response to intra-VTA ghrelin was not significantly different between chow and HFD fed mice. Our results also suggest that diet-induced obesity impairs metabolic sensing in ghrelin-resistant hypothalamic structures influencing mesolimbic dopamine pathways. Presumably ghrelin is part of the metabolic signal that communicates homeostatic information to mesolimbic dopaminergic pathways (Lockie and Andrews, 2013). Our results, together with previously published reports of ghrelin resistance in NPY/AgRP neurons (Briggs et al., 2010, 2014) support the concept that DIO broadly initiates ghrelin resistance in the brain. The physiological relevance of ghrelin resistance may represent a mechanism that protects a higher body weight set point established by HFD. Indeed, ghrelin resistance does not prevent weight gain on a HFD (Perez-Tilve et al., 2011), but rather protects against diet-induced weight loss when ghrelin sensitivity is reinstated upon reducing calorie intake (Briggs et al., 2013). The presence of ghrelin resistance in both the homeostatic and reward-processing systems suggests diet-induced weight loss will be rapidly countered by both systems, defending against excessive weight loss. Understanding these mechanisms may help design treatment strategies that maximise diet-induced weight loss and prevent rebound weight gain often seen in chronic dieters.

Conflict of interest

The authors declare no conflict of interest and have nothing to disclose.

Disclosure

The authors have nothing to disclose.

Contributors

S.H.L., A.J.L. and Z.B.A. designed experiments; S.H.L., T.D., S.J.S. performed experiments; S.J.S., A.J.L. edited manuscript; S.H.L. and Z.B.A. wrote manuscript.

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